ORIGINAL ARTICLE

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Interferon alpha-induced reduction in the values of myeloid-derived suppressor cells in melanoma patients

Sniženje vrednosti supresorskih ćelija mijeloidnog porekla kod bolesnika sa melanomom indukovano interferonom alfa

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Abstract

Background/Aim. Interaction between tumor cells and host's immunoregulatory cells in creation of microenvironment that supports tumor progression is the focus of numerous investigations in recent years. Myeloid-derived suppressor cells (MDSCs) are heterogeneous population of immature dendritic cells, macrophages and granulocytes. In cancer patients, these cells accumulate in tumor microenvironment, tumor-draining lymph nodes, peripheral blood and the liver and their numbers correlate with the stage of the disease and the metastatic disease. The aim of the study was to investigate the effect of interferon alpha on MDSCs percentage in peripheral blood of melanoma patients. **Methods.** The interferon treated melanoma patients were given subcutaneously interferon alpha, in optimal dose, for a period of at least 6 months before the analysis. Blood samples were collected from the melanoma patients (n = 91) and the age/sex matched healthy controls (n = 8). The following anti-human monoclonal antibodies were used for immunostaining: anti-CD15-FITC, anti-CD33-PE, anti-CD45-ECD, anti-HLA-DR PE/Cy5, anti-CD14-FITC, anti-CD16-PE and anti-CD11b-PE. **Results.** Comparison of myeloid-derived suppressor cells values in the stage 2 melanoma patients with and without interferon alpha therapy did not show a significant difference. When we compared the MDSCs values in the patients within stage 3 melanoma, we found a significant difference in granulocytic subset values between the interferon alpha-treated and the untreated group. Comparison of values of all suppressor cells populations between the interferon alpha-treated patients and healthy controls showed a significant increase in suppressor cells percentage in the melanoma patients. The granulocytic and total MDSCs values were significantly lower in the interferon alpha treated melanoma patients with progression in comparison with untreated patients with stable disease. **Conclusion.** We confirmed that interferon alpha effect in stage 3 melanoma patients was reduction in MDSCs percentage. We also found an unexpected bounce back of these suppressor cells levels, many months after the discontinuation of interferon alpha therapy.

Key words:
melanoma; myeloid cells; interferon-alpha.

Apstrakt

Uvod/Cilj. Interakcija između tumorskih ćelija i imunoregulatornih ćelija domaćina u stvaranju mikrookruženja koje pospešuje progresiju tumora nalazi se u žiži brojnih istraživanja poslednjih godina. Supresorske ćelije mijeloidnog porekla predstavljaju heterogenu populaciju nezrelih dendritičnih ćelija, makrofaga i granulocita. Kod bolesnika sa tumorom ove ćelije akumuliraju se u tumorskom mikrookruženju, drenažnim limfnim čvorovima, perifernoj krvi i jetri i njihov broj koreliše sa stadijumom bolesti i metastatskom bolešću. Cilj rada bio je ispitivanje efekata interferona alfa na procentualnu zastupljenost supresorskih ćelija mijeloidnog porekla u perifernoj krvi bolesnika sa melanomom. **Metode.** Bolesnici lečeni interferonom dobijali su interferon alfa potkožno, u optimalnim dozama, najmanje šest meseci pre izvođenja analize. Uzorci krvi uzimani su od bolesnika sa melanomom (n = 91) i zdravih kontrola (n = 8) sličnog uzrasta i pola. Sledeća antihumana monoklonska antitela korišćena su za imunofenotipizaciju: anti-CD15-FITC, anti-CD33-PE, anti-CD45-ECD, anti-HLA-DR PE/Cy5, anti-CD14-FITC, anti-CD16-PE i anti-CD11b-PE. **Rezultati.** Poređenjem vred-

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nosti supresorskih ćelija mijeloidnog porekla između bolesnika u 2. stadijumu melanoma koji jesu i bolesnika koji nisu lečeni interferonom alfa, nije utvrđena statistički značajna razlika. Kada smo uporedili vrednosti supresorskih ćelija mijeloidnog porekla kod bolesnika u 3. stadijumu melanoma pronašli smo značajnu razliku u vrednostima granulocitne podgrupe ovih ćelija između grupe lečenih i grupe nelečenih interferonom alfa. Poređenjem vrednosti svih populacija ovih supresorskih ćelija između bolesnika lečenih interferonom alfa i zdravih osoba utvrđene su značajno više vrednosti supresorskih ćelija kod bolesnika sa melanomom. Granulocitne i ukupne supresorske ćelije mijeloidnog porekla bile su značajno niže kod bolesnika sa progresijom melanoma koji su lečeni interferonom alfa nego kod bolesnika sa stabilnom bolešću koji nisu lečeni interferonom alfa. **Zaključak.** Interferon alfa dovodi do sniženja vrednosti supresorskih ćelija mijeloidnog porekla kod bolesnika u 3. stadijumu melanoma. Takođe, utvrdili smo povratak visokih vrednosti ovih supresorskih ćelija nakon mnogo meseci od prestanka terapije interferonom alfa.

Ključne reči:
melanom; ćelije, mijeloidne; interferon-alfa.

Introduction

Although malignant melanoma comprises < 5% of all malignant skin tumors it is responsible for almost 60% of lethal skin neoplastic diseases [1]. In the World Health Organisation (WHO) classification there are 4 common types of melanomas (superficial spreading, nodular, lentigo maligna and acral lentiginous) and 6 less frequent types (desmoplastic, melanoma arising from a blue nevus, melanoma arising in a congenital nevus, melanoma of childhood, nevoid melanoma and persistent melanoma) [2]. A typical patient is a Caucasian in the 4th decade of life and the most common locations are on the back in males and the leg in females. Risk factors for developing melanoma are pale skin, blond or red hair, numerous freckles and tendency to burn and tan poorly, the presence of more than 50 acquired nevi, > five dysplastic nevi, chemical exposures, immunosuppression, scars, genetic factors etc. Intermittent sun exposure is recognized as the most important factor [1].

The risk of recurrence after surgical removal of primary tumor, for stage IIB and stage III melanoma patients is reported to be approximately 60% and 75%, respectively [3], so the need for adjuvant therapy is obvious. Malignant melanoma is an immunogenic tumor, susceptible to attack by the host's immune system [4] and, therefore, a broad spectrum of immunotherapies was developed. Unfortunately, many of the tested agents (nonspecific immunostimulants, vaccine and cytokine therapies) failed to demonstrate significant clinical impact. Malignant melanoma is known for its aggressive behavior that is caused by various factors including certain immunosuppressive and immunomodulating molecules released by host cells and melanoma cells [interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), NO, matrix metalloproteinases (MMPs)], tumor editing and other escape mechanisms [5]. Interaction between tumor cells and host's immunoregulatory cells in creation of microenvironment that supports tumor progression is the focus of numerous investigations in recent years. Beside a well-known regulatory T lymphocytes (Tregs), myeloid-derived suppressor cells (MDSCs) function as suppressors of an anti-tumor immunity. Both cell types are involved in development of malignant melanoma [6, 7].

MDSCs are a heterogeneous population of immature dendritic cells, macrophages and granulocytes. In mice, they are identified by CD11b+, IL-4Rα+ and Gr1+ expression. The same cell population is less well defined in humans, but in general MDSCs are myeloid derived (CD33+), CD11b+, lineage not determined (Lin-: CD3-, CD19-, CD56-, CD14-), suppressive and with poor antigen presenting function (HLA-DR-/low). In healthy people they are rare or absent, but under some circumstances (trauma, sepsis) may accumulate in order to temper immune response. In cancer patients, MDSCs accumulate in the tumor microenvironment, tumor-draining lymph nodes, peripheral blood and the liver. Their number correlates with the stage of the disease and the metastatic disease [8]. The influence of MDSCs on anti-tumor immune response is strong and comprehensive, hence these cells are an excellent target in fighting strategies against tumors such as: stimulation of differentiation MDSCs into mature non-suppressive phenotype, decreasing numbers of MDSCs, and inhibition of suppressive function of MDSCs on anti-tumor immunity [9–11]. MDSCs play an important role in melanoma progression and/or as a predictive test for the response to immune-therapy. Finkelstein et al. [12] showed that melanoma and renal cell carcinoma patients with low MDSCs values and a high dendritic cells/MDSCs ratio significantly better responded to high dose IL-2 therapy [12]. The evidence of significant role of MDSCs in melanoma development is accumulating [13].

Interferons demonstrate diverse effects on tumor cells and, between others, interferon alpha (IFNα) showed the highest degree of activity against melanoma cells. Although the precise mechanisms of action are not well understood, antitumor effects of IFNα could include direct anti-proliferative effects, the enhancement of natural killer (NK) cells activity and the up-regulation of tumor antigens and/or major histocompability complex (MHC) class I and class II molecules expression [14]. Early trials with adjuvant IFNα therapy showed significantly longer relapse-free and overall survival rates in melanoma patients [15]. Based on the study of Kirkwood et al. [15], the U.S. Food and Drug Administration (FDA) approved the use of postsurgical adjuvant therapy of high-risk melanomas and this was widely adopted in the community as the best standard of care [16]. Subsequent trials with IFNα showed controversial results [17].

The IFNα effects on MDSCs could be a consequence of induction of maturation in these immature suppressive cells. In addition to lowering the number of MDSCs, IFNα therapy also leads to inhibition of their suppressive activity *in vitro*, as shown in the study of Zoglmeier et al. [18]. Lower suppressive activity of MDSCs under the influence of IFNα therapy could be the consequence of reduced arginase activity and reduced production of reactive oxygen species by MDSCs.

The correlation of IFNα therapy with MDSCs and Tregs levels in peripheral blood of melanoma patients was examined in more detail by Tarhini et al. [19] in 2012 who showed a significant decrease of MDSCs percent in peripheral blood of melanoma patients on day 29 from the beginning of IFNα therapy (after completion of the induction phase of IFN) and day 85 (after completion of one course of IFNα therapy in combination with anti-CTLA-4 antibody).

The IFNα therapy effects on MDSC amount in peripheral blood are noted during therapies of some other diseases, particularly in chronic hepatitis C virus (HCV) infection. Mohamed et al. [20] showed significantly lower MDSC values in patients with chronic HCV infection who had good response to IFNα therapy when compared with patients who had poor response to IFNα.

The aim of this study was to investigate the effect of IFNα on MDSCs percentage in peripheral blood of melanoma patients.

Methods

Patients and healthy controls

Malignant melanoma patients were recruited for this study from the Clinic for Dermatovenerology and Clinic for Plastic and Reconstructive Surgery of the Military Medical Academy (MMA) in Belgrade. Healthy controls were recruited from periodical systematic examinations of apparently healthy persons, with no prior history of cancer. All patients and healthy controls were consented and this study was approved by the local Research Ethics Committee. Melanoma patients were classified according to the 7th edition of the American Joint Committee on Cancer (AJCC) classification for melanoma [21, 22].

IFNα dosage and recorded parameters

All IFNα treated melanoma patients were given subcutaneously 10×10^6 IU five times per week for one month (induction), followed by maintenance regime in optimal dose according to age and stage of the disease (range 3 to 6 × 106 IU) three times *per* week. The patients were on treatment for at least 6 months before the analysis was carried out. Follow-up examinations were repeated every three-months. The parameters were obtained by clinical and dermoscopic examination, laboratory analyses: complete and differential blood count, general biochemical analyses, lactate dehydrogenase (LDH) and S100A protein, ultrasound examination of regional lymph nodes, radiographic and periodic MSCT imaging.

Samples

Three to six milliliters of venous blood were collected from 91 melanoma patients whose age/sex was matched with 8 healthy controls in the period between October 2012 and December 2012. Blood samples were drawn into 3 milliliters vacuettes with Na-EDTA. Erythrocytes were removed with lysing buffer (EDTA, NH_4Cl, $KHCO_3$) for 10 minutes with constant mixing. Remaining nucleated cells were washed twice in RPMI 640 medium with 5% of normal human serum, by standard centrifuge and resuspension processes. The cells were counted both manually, in improved Neubauer chamber, and automatically on Beckman Coulter ACT differ blood counter, and 1×10^6 cells/100 μL of suspension was aliquoted in 12 × 75 mm test tubes for further immunostaining.

Immunophenotypic analysis of cells

The following anti-human monoclonal antibodies were used for immunostaining of fresh peripheral blood samples: anti-CD15-FITC, anti-CD33-PE, anti-CD45-ECD, anti-HLA-DR PE/Cy5, anti-CD14-FITC, anti-CD16-PE, anti-CD11b-PE, anti-CD3-FITC, anti-CD19-FITC and anti-CD56-FITC (Beckman Coulter), in a different combination for multicolor analysis. Stained cells were analyzed using Beckman Coulter FC 500 flow cytometer with CXP analysis software. MDSCs were defined as lineage negative (CD3-, CD19-, CD56-, CD14-), HLA-DR-/low, CD11b+ and CD33+ cells. They were primarily gated on CD11b Vs. HLA-DR plot. The cells with negative/low expression of HLA-DR and positive for CD11b, were further analyzed for lineage markers, CD15 and CD45 expression. Detection of granulocytic and monocytic subsets was made on the basis of CD15 and CD14 expression, respectively. MDSCs percentages were expressed as percent of all nucleated cells.

Statistical analysis

Data analysis was performed using GraphPad Prism 5 software using unpaired, two tailed Student *t*-test for analysis of two groups, and one-way ANOVA test for analysis of multiple groups

Results

MDSCs values in the IFNα treated and untreated melanoma patients

The values of MDSCs were determined in 91 melanoma patients grouped according to the AJCC classification for melanoma. Eleven out of these 91 patients were at active IFNα therapy at the time of MDSCs analysis, and all of them were in the AJCC stage 2 or stage 3. The AJCC subclassification (2a, 2b, 2c, 3a, 3b, 3c) could not be used for statistical analysis because of the small number of patients within each sub-stage.

Comparison of two MDSCs populations, both granulocytic subset of MDSCs (GrMDSCs) and total MDSCs between IFNα treated and untreated melanoma patients did not bring any significant difference, regardless of the AJCC stage (Figure 1). Comparison of GrMDSCs and total MDSCs values in stage 2 melanoma patients with and without IFNα therapy did not show any significant difference (data not shown). However when we compared the MDSCs values in the patients within AJCC stage 3 melanoma, we found a significant difference in GrMDSCs values between the IFNα treated and untreated group. Yet, there was no real significance observed in the total MDSCs values in patients within the AJCC stage 3 (Figure 2).

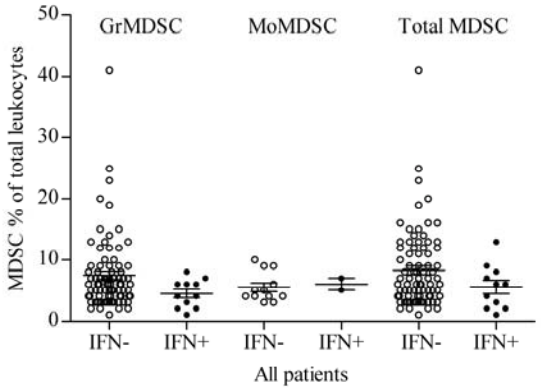

Fig. 1 – Myeloid-derived suppressor cells (MDSCs) values of the American Joint Committee on Cancer (AJCC) stage III melanoma patients, interferon (IFNα)-treated (IFN+) and untreated (IFN-).

The frequency of granulocytic subset of MDSCs (GrMDSCs), monocytic subset of MDSCs (MoMDSCs) and total MDSCs was compared between all the IFN+ (n = 11) and all IFN- (n = 80) melanoma patients regardless of the AJCC classification, using unpaired two-tailed Student's *t*-test, and there was no significant differences. The values are given as mean ± standard error of the mean (SEM).

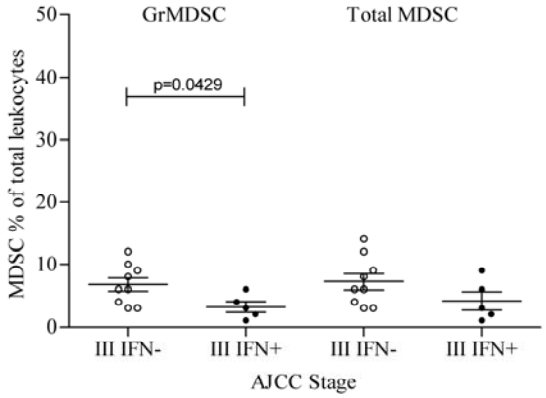

Fig. 2 – Myeloid-derived suppressor cells (MDSCs) values (Gr, Mo and total MDSCs populations) in the interferon alfa (IFNα)-treated (IFN+) and untreated (IFN-) melanoma patients, regardless of the American Joint Committee on Cancer (AJCC) classification.

The frequency of granulocytic subset of MDSCs (GrMDSCs) and the total MDSCs was compared between IFN+ melanoma pateints in the AJCC stage III (n = 6) and IFN- (n = 9) melanoma patients in the AJCC stage III, using unpaired two tailed Student's *t*-test, and difference in frequency of GrMDSCs was significant ($p = 0.049$). The values are given as mean ± standard error of mean (SEM).

Examination of monocytic subset of MDSCs (MoMDSCs) between patients in different AJCC stages was not possible because of the small number of patients with detectable levels of this subset within single stages of melanoma. GrMDSC values in peripheral blood of stage 3 melanoma patients at IFNα therapy were significantly lower than GrMDSC values of stage 3 melanoma patients without IFNα therapy.

MDSCs values in IFNα treated melanoma patients and healthy controls

Comparison of values of all MDSCs populations between IFNα treated patients and healthy controls showed a significant increase in GrMDSCs, MoMDSCs and total MDSCs numbers in melanoma patients samples (Figure 3).

Disease progression and MDSCs values in the IFNα treated and untreated melanoma patients

The 22 out of 91 melanoma patients showed progression of the disease (advance to the next stage, local reccurrence of melanoma within the same stage). The 22 patients with melanoma progression were further classified in two groups: the group under IFNα therapy (n = 6) and without IFNα therapy (n = 16).

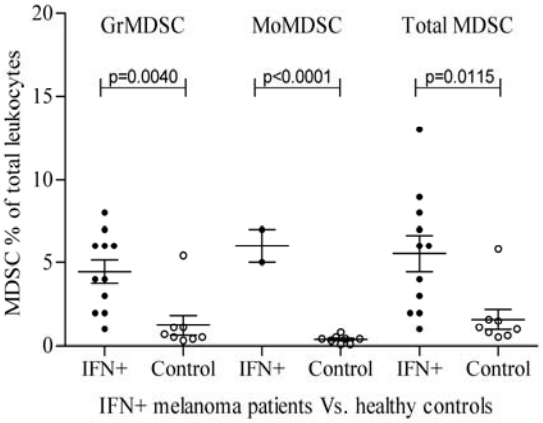

Fig. 3 – Myeloid-derived suppressor cells (MDSCs) values of the interferon (IFNα)-treated (IFN+) melanoma patients and the healthy controls.

The frequency of granulocytic subset of MDSCs (GrMDSCs), monocytic subset of MDSCs (MoMDSCs) and the total MDSCs was compared between all the IFN+ melanoma patients (n = 11) and the healthy controls (n = 8), using unpaired two-tailed Student's *t*-test, and differences in frequency of GrMDSCs, MoMDSCs and the total MDSCs were significant ($p = 0.0040$, $p < 0.0001$, $p = 0.0115$, respectively). The values are given as mean ± standard error of the mean (SEM).

Both groups of patients were compared for all MDSCs values with the following results. There was no statistically significant difference in GrMDSCs and total MDSCs (data not shown). When we excluded extreme values, we found a significant difference in GrMDSCs percentage between IFNα trea-

ted and untreated melanoma patients with progressive disease (Figure 4). Again, the total MDSCs number did not differ significantly between the two examined groups even after exclusion of extreme values. Examination of the MoMDSCs subset was not possible because of the small number of patients with detectable levels of this subset. The most important findings were significantly lower values of GrMDSCs in the patients with melanoma progression who were on IFNα therapy *versus* those with melanoma progression without IFNα therapy.

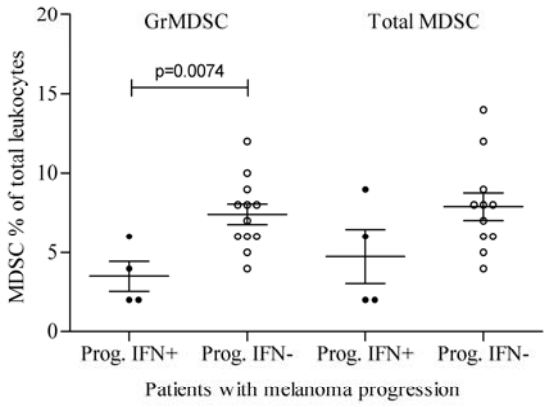

Fig. 4 – Disease progression and myeloid-derived suppressor cells (MDSCs) values in the interferon (IFNα)-treated (IFN+) and untreated (IFN-) melanoma patients.
The frequency of GrMDSCs and the total MDSCs was compard between the IFNα treated melanoma patients with progressive disease (Prog.IFN+, n = 6) and the IFNα untreated melanoma patients with progressive disease (Prog.IFN-, n = 16), using unpaired two tailed Student's *t*-test, and difference in frequency of GrMDSC was significant (p = 0.0074). The values are given as mean ± standard error of the mean (SEM).

MDSCs values in the IFNα untreated patients, with and without melanoma progression

On the basis of two criteria, advancing to the next stage of the disease and local reccurrence of melanoma within the same stage 22 of 91 patient were classified in the group of those with melanoma progression, 55 patients comprised the group of patients with stable disease, while for the 4 of 91 patients there was no sufficient clinical data to determine progression status and they were excluded from the analysis. This classification was made regardless of clinical and pathohistological stage at the time of diagnosis. Within the group of patients with melanoma progression, 15 of 22 patients were IFNα untreated, while in the group of patients without progression, 50 of the 55 patients were IFNα untreated, and the MDSC values were compared between these two groups. We found that the patients with melanoma progression had significantly higher GrMDSCs values (p = 0.0475) than the patients without melanoma progression (Figure 5). With additional statistical processing, by exclusion of extreme values, we found statistically highly significant differences in GrMDSC (p = 0.0034) and total MDSC (0.0051) values between the two groups (Figure 6). The MoMDSCs subset was detectable in 11 patients with stable disease and 3 patients with melanoma progression, and we did not find any statistically significant difference between the two groups in the values of this MDSCs subset (Figure 5).

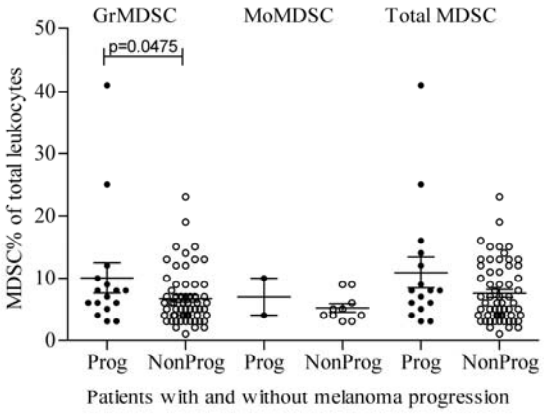

Fig. 5 – Myeloid-derived suppressor cells (MDSCs) values in the interferon (IFNα)-untreated patients with (Prog) and without (NonProg) melanoma progression.
The frequency of granulocytic subset of MDSCs (GrMDSCs), monocytic subset of MDSCs (MoMDSCs) and the total MDSCs was compared between the IFNα-untreated melanoma pateints with progressive disease (Prog, n = 15) and the IFNα-untreated melanoma pateints without disease progression (NonProg, n = 50), using unpaired two tailed Student's *t*-test, and the difference in frequency of GrMDSC was significant (p = 0.0475). The values are given as mean ± standard error of the mean (SEM).

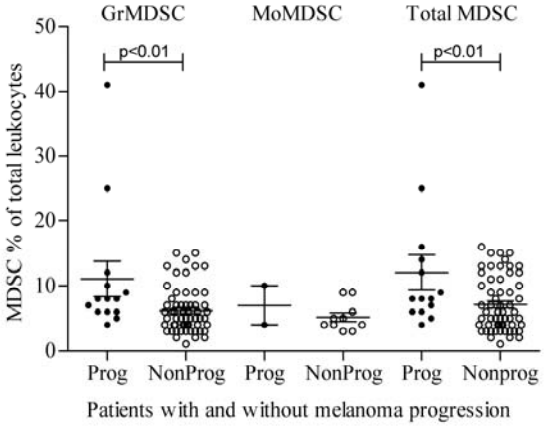

Fig. 6 – Myeloid-derived suppressor cells (MDSCs) values in the interferon (IFNα)-untreated patients with (Prog) and without (NonProg) melanoma progression (extreme values excluded).
The frequency of granulocytic subset of MDSCs (GrMDSCs), monocytic subset of MDSCs (MoMDSCs) and total MDSCs was compared between the IFNα untreated melanoma pateints with progressive disease (Prog, n = 15) and IFNα untreated melanoma pateints without disease progression (NonProg, n = 50) regardless of the American Joint Committee on Cancer (AJCC) classification, using unpaired two-tailed Student's *t*-test, and the differences in frequency of GrMDSC and total MDSCs were significant (p = 0.0034 and p = 0.0051, respectively). The following extreme values were excluded: ID876 = 1%, ID964 = 19% and ID973 = 23% within the group of patients without progression, and ID949 = 3% within the group of patients with melanoma progression. Values are given as mean ± standard error of the mean (SEM).

The values of GrMDSCs and total MDSCs were significantly higher in the group of patients with melanoma progression when compared with the group of patients with

stable disease, while the values of MoMDSCs did not show any statistically significant difference.

MDSCs values in the IFNα treated patients with disease progression and the IFNα untreated patients without melanoma progression

On the basis of two criteria, disease progression and application of IFNα therapy, our melanoma patients were classified in two groups. The group I comprised of patients without melanoma progression and without IFNα therapy (n = 61), while the group II comprised of patients with progressive melanoma disease who were on IFNα therapy at the time of analysis (n = 6). Comparision of these two groups showed a significantly lower GrMDSCs and total MDSCs values in the patients with melanoma progression and IFNα therapy, *versus* the group of patients without melanoma progression and without IFNα therapy (Figure 7). Examination of the MoMDSCs subset was not possible because of a small number of patients with detectable levels of this subset.

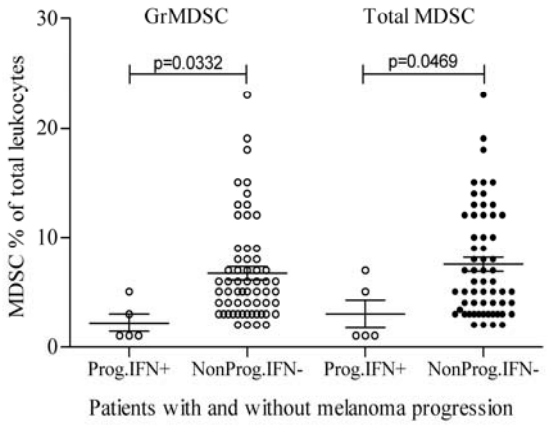

Fig. 7 – Myeloid-derived suppressor cells (MDSCs) values in the patients without melanoma progression and without IFNα therapy (NonProg.IFN-) *versus* the patients with melanoma progression and with IFNα therapy (Prog.IFN+).
The frequency of granulocytic subset of MDSCs (GrMDSCs), monocytic subset of MDSCs (MoMDSCs) and total MDSCs was compared between the IFNα-untreated melanoma patients with stable disease (NonProg.IFN-, n = 61) and IFNα-treated melanoma patients with progressive disease (Prog.IFN+, n = 6) regardless of the American Joint Committee on Cancer (AJCC) classification, using unpaired two tailed Student's *t*-test, and differences in frequency of GrMDSCs and total MDSCs were significant ($p = 0.0332$ and $p = 0.0469$, respectively). The values are given as mean ± standard error of the mean (SEM).

The obtained results show that GrMDSCs and the total MDSCs values were significantly lower in the melanoma patients with progression and at IFNα therapy than in the melanoma patients without disease progression and without IFNα therapy.

Discussion

We compared MDSC values in the two groups of melanoma patients irrespective of the stage. One group was treated with IFNα and the other was not. We found that the MDSCs values for these two groups did not show a significant difference. When we analyzed MDSCs values in all melanoma patients separated in groups by melanoma stage, we found a trend of increase in MDSCs numbers with stage progression. MDSCs values in the stage IV melanoma patients were significantly higher compared to all other stages, however there was no statistical significance between the successive melanoma stages (I-III) (data not shown).

Comparison of MDSCs values in the IFNα treated and untreated groups for each stage showed significant differences for the stage III melanoma patients. The melanoma patients with IFNα therapy had significantly lower GrMDSCs values. IFNα therapy has already been implemented into national guidelines for the treatment of stage III melanoma patients in many European countries [23, 24]. In a large study which comprised 1,256 patients with resected stage III melanoma, Eggermont et al. [25] showed that adjuvant pegylated interferon alfa-2b had a significant, sustained effect on recurrence-free survival. Sondak and Flaherty [26] emphasized that in the Eggermont's study, patients with micrometastases in sentinel lymph node, had the strongest benefit from IFNα therapy.

In our study, 4 of 6 (67%) patients within stage III melanoma at IFNα therapy, had micrometastases in sentinel lymph nodes. This finding implies comparison of MDSCs values in IFNα-treated patients with micrometastases *versus* IFNα-treated patients with macrometastases, in order to investigate eventual correlation of the above mentioned therapy benefit with the reduction of MDSCs levels.

Kimberly et al. [27] showed that MDSCs levels correlate with the disease progression in melanoma patients. Our patients with progressive disease without IFNα therapy had higher MDSCs values in peripheral blood in comparison with the group of patients with stable disease, also without IFNα therapy. We showed that IFNα-treated melanoma patients with progressive disease had significantly lower values of MDSCs than those with no IFNα therapy. In IFNα treated patients with progressive disease MDSCs reduction was very marked, the average MDSCs number was lower than a corresponding value in the patients with stable disease. Again, comparison of MDSCs values from patients with progressive disease at IFNα therapy with those with stable melanoma disease who were without IFNα therapy, showed significantly lower MDSCs values in patients with progressive disease at IFNα therapy at the time of analysis.

Unexpectedly, 2 of our melanoma patients (IDs 956 and 958), had the history of IFNα therapy prior to entering the study, with their therapy being finished more than 24 months before MDSCs measurements hence they were classified as patients without IFNα therapy. In these 2 patients MDSCs values were extremely high, 14% and 20% of total leukocytes, respectively, raising the question on long-term effects after IFNα therapy cesation. Also, the time from discontinuation of IFNα to MDSCs level measurement is 4 times longer in our study than in the study of Mohamed et al. [20] who showed that 4–6 months after IFNα treatment MDSCs values in hepatitis C patients with good response to IFNα therapy were

significantly lower than the values obtained during active treatment in the same patients. Finally, Mohamed et al. [20] measured MDSCs values in HCV patients, so the studies could not be directly compared. Our findings show that long-term effects, after discontinuation of IFNα therapy, on MDSCs levels in peripheral blood may be the opposite from expected and this deserves further investigations. Essentially there could be a significant bounce back of MDSCs levels, many months after discontinuation of IFNα, resulting in greater numbers than would normally be found.

When we compared MDSCs values in all the melanoma patients at IFNα therapy at the time of the analysis with MDSCs values in the healthy controls not subjected to INFα, we found significantly higher values of GrMDSCs, MoMDSCs and the total MDSCs in the IFNα-treated group. So, although IFNα therapy showed significant effects on MDSCs levels in peripheral blood of melanoma patients, MDSCs levels in patients receiving IFNα therapy could not be decreased to the levels of MDSCs in healthy controls.

Conclusion

This study confirmed that the effect of IFNα in stage III melanoma patients was the reduction in MDSCs percentage. IFN therapy must be considered when analyzing MDSCs values in peripheral blood. We also found an unexpected bounce back of MDSCs levels, many months after the discontinuation of IFNα therapy in melanoma patients.

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